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(54) Title: TREATMENT OF DISORDERS USING POLYETHYLENIMINE DIAZENIUMDIOLATE

(57) **Abstract:** A method for the treatment of a patient having, or susceptible to having, symptoms of a disorder or dysfunction comprises administering a non-fibrous linear poly(ethylenimine) diazeniumdiolate to the patient wherein said linear poly(ethylenimine) diazeniumdiolate is capable of releasing a therapeutically effective amount of nitric oxide sufficient to alleviate the symptoms of the disorder or dysfunction. A method for delivering nitric oxide to the lungs comprises the steps of synthesizing a non-fibrous linear poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of N_2O_2- substituted amines to unsubstituted amines greater than 1.0; and administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs. A method for lowering the pulmonary blood pressure of a patient having lungs, without significantly affecting the systemic vascular blood pressure of the patient comprises the steps of synthesizing a non-fibrous linear poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of N_2O_2- substituted amines to unsubstituted amines greater than 1.0; and administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs.

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TREATMENT OF DISORDERS USING POLYETHYLENIMINE DIAZENIUMDIOLATE

BACKGROUND OF THE INVENTION

This invention relates to the treatment of disorders and dysfunctions in patients. More particularly, this invention relates to the use of nitric oxide releasing compounds to treat or otherwise alleviate the symptoms of disorders or dysfunctions. Specifically, this invention relates to the use of non-fibrous linear polyethylenimine diazeniumdiolate as a nitric oxide releasing compound in the treatment of these disorders and dysfunctions.

The importance of nitric oxide (NO) in biological repair mechanisms is well known even though the precise mechanism of its action has not been completely elucidated. Among its other effects, NO is known to inhibit the aggregation of platelets and to reduce smooth muscle proliferation, which is known to reduce restenosis. When delivered directly to a particular site, it has been shown to prevent or reduce inflammation at the site where medical personnel have introduced foreign objects or devices into the patient. NO is also known to reduce systemic blood pressure.

Researchers have sought various ways to deliver NO to damaged tissue and to tissues and organs at risk of injury. NO can be delivered systemically, but such delivery can bring undesired side effects with it. Ideally, NO should be delivered in a controlled manner which is directed specifically to those tissues and organs that have been injured or are at risk of injury. Various compounds have been used to deliver NO therapeutically. Among these are classes of NO donors that either require activation to release therapeutic levels of nitric oxide, or they release both NO and undesired free radicals. Examples of such classes of NO donors include sydnonimines such as, for example, molsidomine. Other compounds which have been used for delivery of NO to a specific site include metallocorrinoids, such as a vitamin B_{12} compounds, as described in U.S. Patent No. 5,936,082, and chitosan polymers which are described in co-pending U.S. Patent

Application No. 09/199,732 filed on November 25, 1998.

Diazeniumdiolates (NONOates) are a class of compounds that contain at least one $N_2O_2^-$ group and exhibit the ability to release NO spontaneously under physiological conditions without the release of free radicals. Polymeric NONOates may be formed by attaching the $N_2O_2^-$ group directly onto a polymeric backbone. Water insoluble polymeric NONOates (e.g., polyethylenimine cellulose diazeniumdiolate)and their use to accelerate wound repair have been generally described in U.S. Patent No. 5,519,020. These polymers were used to deliver NO to specific tissues, and results have shown that controlled release of NO to a specific site greatly reduces inflammation and accelerates the healing process at that site. However, these compositions heretofore have had to be delivered by topical application.

More recently, polymers such as poly(ethylenimine) (PEI) have been used as a backbone for the attachment of $N_2O_2^-$ and delivery of NO to particular sites and under specific conditions to treat specific diseases. Attachment of $N_2O_2^-$ to PEI creates a polyethylenimine diazeniumdiolate (PEI-NO).

The use of a form of PEI-NO to deliver NO has been previously described in U.S. Patent No. 5,714,511. Sepsis, which is the presence of pathogenic bacteria or their products in the bloodstream, can cause damage to tissues and organs either directly or indirectly. Indirect mechanisms are those associated with inflamation, and can occur regardless of the strain or pathogenicity of the infecting bacteria. NO has been identified as providing a protective effect with regard to sepsis and its end result, septic shock. Patent No. 5,714,511 describes a method for delivering NO to tissues that are at risk of injury due to sepsis or shock using various protected diazeniumdiolates. The protecting group prevents or at least limits the systemic release of nitric oxide from the diazeniumdiolate. Preferred protecting groups are those that can be metabolically removed from the diazeniumdiolate by the tissue or organ of interest, namely the liver or the kidneys. The removal of the protecting group allows the NO of the diazeniumdiolate to then be delivered. By removing the protecting group within the target tissue or organ, NO is preferentially delivered to that tissue or organ, thereby

preventing both dilution of the NO systemically and chemical destruction of the NO prior to delivery to the target tissue or organ. Among the polymers which may be used to deliver NO to specific tissues, such as the liver or the kidneys, is protected polyethylenimine diazeniumdiolate. A need exists, however, for a method for the delivery of NO to these and other specific tissues or organs that is not dependent on removal of a protecting group or other method of activation or metabolization of a NO-delivering prodrug.

While such uses of NONOates have been successful for the above mentioned uses, these methods have not been useful for the specific administration of NO to areas or internal organs that are inaccessible to topical application, such as the lungs, without activation. Furthermore, the usefulness of topical application of PEI-NO has been limited due to the relatively low level of NO substitution on previously known branched PEI. Branched poly(ethylenimine), or BPEI, is a branched polymer with repeating (NCH_2CH_2) groups, branched N-containing tertiary amines, and end groups containing primary amines. The ratio of primary amines to secondary amines to tertiary amines is generally 1:2:1 in BPEI. Because NO forms stable diazeniumdiolate products only with the secondary amines in poly(ethylenimine), the NO:polymer ratio is relatively low for BPEI diazeniumdiolates. Additionally, substitution of the available secondary amines in BPEI with NO may not be complete in all cases, thereby lowering the amount of NO available for delivery further.

As mentioned above, the use of NO to reduce systemic blood pressure is known. The use of various compounds to deliver NO and thus reduce blood pressure is described, for example, in U.S. Patent Nos. 5,039,705, 5,155,137, 5,212,204, and 5,405,919. In treating certain disorders, however, it may be desirable to reduce pulmonary blood pressure selectively, i.e., without reducing systemic blood pressure. Currently, pulmonary blood pressure is reduced by delivering NO gas to the lungs. Use of NO gas to treat pulmonary disorders, however, is less than ideal. The use of NO gas requires the on-site storage of tanks of pressurized NO gas. This brings with it all of the hazards associated with the use of any pressurized gas, including accidental release of the gas, either slowly or catastrophically. Additionally, upon contact with air, nitric oxide

can be converted to nitrogen dioxide, nitrogen tetraoxide, or both. Therefore, delivery of NO gas must be closely monitored and controlled. Thus, a need for a method of delivering nitric oxide to the lungs without the use of NO gas exists. Ideally, such delivery would take place without the need for activation or metabolization of a NO prodrug and without the creation of free radicals.

The use of linear poly(ethylenimine) diazeniumdiolate fibers to deliver NO to a patient is described in co-pending U.S. Application No. 09/571444 filed on May 16, 2000. Such fibers may be used to form a coating layer of fibers on a medical device, such as a shunt or implant. The use of LPEI-NO fibers, however, is not suitable for use in the treatment of conditions where a soluble carrier of NO is required, such as in the treatment of lung diseases. There is, therefore, a need for a form of PEI-NO that is capable of delivering higher levels of NO than has been previously known that is non-fibrous.

BRIEF SUMMARY OF THE INVENTION

It is, therefore, an aspect of the present invention to provide a method of delivering nitric oxide to a patient.

It is another aspect of the present invention to provide a method for the delivery of NO to a patient, as above, without the need for activation and without the creation of free radical-containing compounds.

It is still another object of the present invention to provide a method for delivering NO directly to a patient in a non-systemic, non-topical manner.

It is yet another aspect of the present invention to provide a method for the delivery of NO to a patient, as above, that is non-fibrous.

It is still another aspect of the present invention to provide a method for delivering NO to the lungs of a patient without the use of NO gas, thereby eliminating the

need for storage of pressurized tanks of NO gas for the treatment of such disorders as, for example, pulmonary hypertension, asthma, and emphysema.

It is yet another aspect of the present invention to provide a method for reducing the pulmonary blood pressure of a patient without reducing the systemic blood pressure of the patient.

It is still another aspect of the present invention to provide a non-fibrous linear poly(ethylenimine) diazeniumdiolate which has a greater degree of $N_2O_2^-$ substitution than previously known branched poly(ethylenimine) diazeniumdiolate.

At least one or more of the foregoing objects, together with the advantages thereof over the known art relating to delivery of NO to a patient, which shall become apparent from the specification which follows, are accomplished by the invention as hereinafter described and claimed.

In general, the present invention provides a method for the treatment of a patient having, or susceptible to having, symptoms of a disorder or dysfunction including administering a non-fibrous linear poly(ethylenimine) diazeniumdiolate to the patient wherein said linear poly(ethylenimine) diazeniumdiolate is capable of releasing a therapeutically effective amount of nitric oxide sufficient to alleviate the symptoms of the disorder.

The present invention also provides a method for delivering nitric oxide to the lungs including synthesizing a non-fibrous linear poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of $N_2O_2^-$ substituted amines to unsubstituted amines greater than 1.0; and administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs.

The present invention also provides a method for lowering the pulmonary blood pressure of a patient having lungs, without significantly affecting the systemic vascular blood pressure of the patient including synthesizing a non-fibrous linear

poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of $N_2O_2^-$ substituted amines to unsubstituted amines greater than 1.0; and administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs.

BRIEF DESCRIPTION OF THE VIEW OF THE DRAWING

The figure is a comparative graph illustrating the changes in the pulmonary vascular resistance and systemic vascular resistance of piglets treated with either LPEI-NO according to the method of the present invention or NO gas.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is directed toward a method of treating disorders with nitric oxide (NO), by producing a linear polyethylenimine diazeniumdiolate (LPEI-NO) in a suitable non-fibrous form for administering the linear polyethylenimine diazeniumdiolate to a patient such that the LPEI-NO is capable of releasing nitric oxide spontaneously under physiologic conditions, without activation or metabolization, and without the creation of free-radical compounds. Synthesis of linear polyethylenimine diazeniumdiolate is accomplished in the following manner. Polyethylenimine may be synthesized by any known method. One such method, for example, is hydrolysis of poly(2-ethyl-2-oxazoline) to form linear poly(ethylenimine). Linear poly(ethylenimine) may also be purchased from a commercial supplier, if available. Linear poly(ethylenimine) is then converted to linear poly(ethylenimine) diazeniumdiolate by exposure of the linear poly(ethylenimine) to nitric oxide.

Non-fibrous linear poly(ethylenimine) diazeniumdiolate is administered in any one of a variety ways, depending on the condition to be treated. Non-fibrous linear polyethylenimine diazeniumdiolate (LPEI-NO) may be administered to a patient alone

or with other medically suitable components, by any of a variety of techniques. These techniques include, for example, oral administration, administration by inhalation, topical administration, and parenteral administration. Linear poly(ethylenimine) diazeniumdiolate may, for example, be incorporated into gels, creams, ointments, sprays, lubricants, aerosols, implants, and patches.

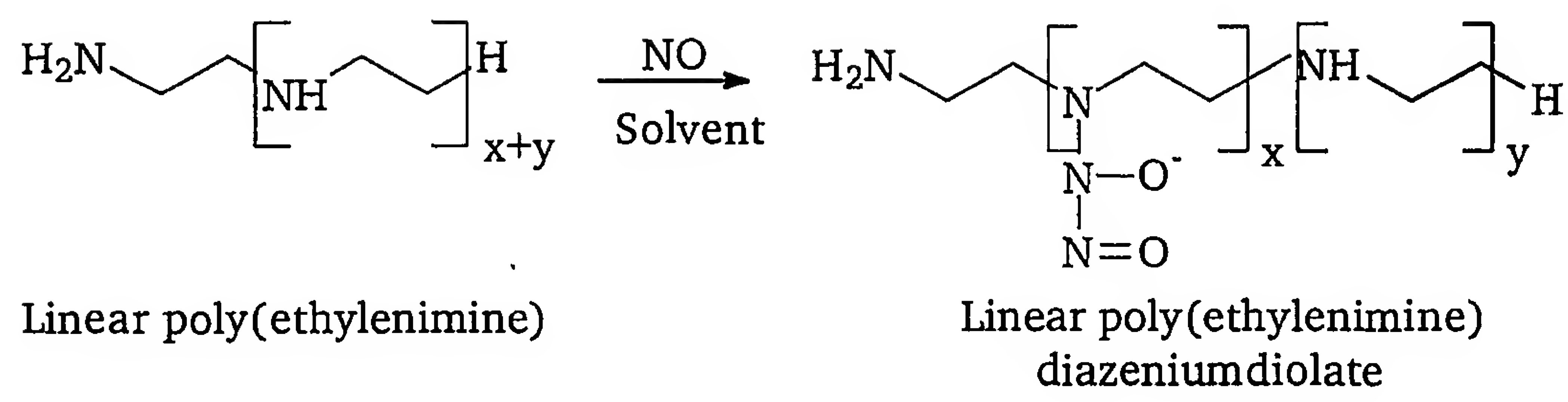
Treatment with a therapeutic level of NO is accomplished by the high ratio of NO:polymer mass afforded by the linear polyethylenimine diazeniumdiolate used in the present invention. Linear poly(ethylenimine) (LPEI) provides superior capacity for NO delivery when converted to PEI-NO compared to branched poly(ethylenimine) (BPEI). As mentioned above, BPEI is a branched polymer with repeating (NCH_2CH_2) groups, branched N-containing tertiary amines, and end groups containing primary amines. The ratio of primary amines to secondary amines to tertiary amines is generally 1:2:1 in BPEI. Because NO is believed to form stable diazeniumdiolate products only with secondary amines, the NO:polymer ratio will be relatively low for BPEI diazeniumdiolates, typically, less than 1. LPEI is substantially free of tertiary amines and, therefore, provides a molecular "backbone" with a higher concentration of secondary amines which are available for substitution with $N_2O_2^-$ than traditional BPEI. Linear poly(ethylenimine) diazeniumdiolate provides a higher potential ratio of NO:polymer mass than previously known branched polyethylenimine diazeniumdiolate, and thereby provides superior NO delivery compared to branched poly (ethylenimine) (BPEI).

The present invention also provides a method of delivering NO at a higher level than has been previously known for non-fibrous forms of PEI-NO. This is accomplished by using LPEI-NO, which can be more highly substituted with NO groups than previously known BPEI. Typically, the degree of substitution afforded by the present invention is at least 25 percent greater than the previously known degree of substitution afforded by BPEI.

The present invention is directed toward the treatment of a patient with a therapeutic level of NO by the administration of non-fibrous linear PEI-NO. Synthesis

of linear polyethylenimine diazeniumdiolate takes place in two steps. The first step is synthesis of linear poly(ethylenimine). Linear poly(ethylenimine) may be prepared by any method known in the art or may be obtained commercially, if available. Among the methods for synthesizing linear poly(ethylenimine) is hydrolysis of poly(2-ethyl-2-oxazoline) disclosed in "Evidence for Long Chain Branching in Polyethyloxazoline," Journal of Polymer Science, Part A, Vol 28, (1990), 3551-3563, the disclosure of which is incorporated herein by reference. Typically, the poly(2-ethyl-2-oxazoline) may have any of a wide range of number average molecular weights, because the molecular weights should not readily affect the formation of linear poly(ethylenimine). Furthermore, the molecular weight may be chosen according to any of a number of factors including the intended use of the final product, as described more fully below.

In the second step of the synthesis of LPEI-NO, LPEI is exposed to nitric oxide. Among the methods useful for converting LPEI to LPEI-NO is exposure of the LPEI to NO under pressure at room temperature as set forth in Scheme I hereinbelow. Suitable solvents and solvent systems typically include organic compounds such as, for example, ether, tetrahydrofuran, methanol, chloroform, methanol, sodium methoxide and mixtures thereof. Preferred solvent systems for the conversion of poly(ethylenimine) to poly(ethylenimine) diazeniumdiolate include chloroform/acetonitrile and methanol/sodium methoxide, although other solvents may be used. The reaction may typically take place under approximately 100 psi (7.031×10^4 kg/m^2) of pressure. The reaction typically takes place at room temperature. The length of time during which the reaction is permitted to take place may vary. Typically, however, the reaction is permitted to proceed for approximately 5 days at room temperature and under approximately 100 psi (7.031×10^4 kg/m^2) of pressure. It is contemplated that any or all of these parameters may vary, however.



(Scheme I)

Upon exposure to NO, the polymer is converted to a diazeniumdiolate derivative of linear poly(ethylenimine). When the reaction has completed, the product is removed from the reaction vessel and the solvent is removed under vacuum, for example, by use of a rotovaporator apparatus. In Scheme I above, x + y is the total number of secondary amines in LPEI, x is the number of $N_2O_2^-$ substituted secondary amines in LPEI-NO, and y is the number of unsubstituted secondary amines in LPEI-NO. The resulting linear polyethylenimine diazeniumdiolate has both $N_2O_2^-$ substituted and unsubstituted secondary amines. In the present invention, the ratio of x:y is at least 1, and preferably, is greater than 1. That is, the method of the present invention may utilize LPEI-NO that contains up to 50 percent unsubstituted secondary amines. More preferably, a majority of the secondary amine sites of a linear poly(ethylenimine) are substituted with $N_2O_2^-$. Most preferably, substantially all of the secondary amine sites of a linear poly(ethylenimine) are substituted with $N_2O_2^-$.

To determine the effectiveness of substitution of NO groups onto the secondary amines of LPEI to form LPEI-NO, the absorbance of the LPEI at 252 nm may be measured. LPEI-NO that is fully substituted with NO at the secondary amines will have an absorbance at 252 nm of between about 6×10^3 m^{-1} and about 8×10^3 m^{-1}. However, it is believed that LPEI-NO that has an absorbance at 252 nm of at least 3×10^3 m^{-1} is effective in delivering a therapeutic level of NO to a patient.

As also shown in Scheme I above, x + y is the total number of secondary

amines in LPEI. In one particular embodiment, the value of x+y is between 8 and 11. In another embodiment, the value of x+y is 9 or 10.

As mentioned above, the linear poly(ethylenimine) component of the LPEI-NO as shown in Scheme I above may be of any molecular weight. The molecular weight selected may vary depending on factors including, for example, the particular application in which the resulting LPEI-NO will be used. In those applications where a water insoluble LPEI-NO is desired, such as those involving topical application of the LPEI-NO, the LPEI used to synthesize LPEI-NO will desirably have a number average molecular weight ranging from about 25,000 to about 200,000, and more preferably, from about 100,000 to about 200,000. In those applications where a water soluble LPEI-NO is desired, such as those applications involving internal use of the LPEI-NO including, for example, inhalation of the LPEI-NO, the LPEI used to synthesize LPEI-NO will desirably have a number average molecular weight of less than about 100,000, more desirably less than about 25,000, and even more desirably, less than about 1,000.

The present invention provides a method of delivering NO to the lungs without the use of NO gas. The present invention, therefore, eliminates the need for storage of pressurized tanks of NO gas for the treatment of lung disorders such as, for example, pulmonary hypertension, asthma, and emphysema. When LPEI-NO is to be administered to the lungs, LPEI-NO may be dissolved in a medically appropriate solvent such as, for example, saline solution, converted into aerosol form by use of pressurized propellant, such as pressurized air or pressurized gas such as nitrogen. LPEI-NO may also be aerosolized without the use of pressurized propellant, for example, by the use of an atomizer or nebulizer. LPEI-NO aerosolized by these or other methods may be administered to the lungs by inhalation either through the mouth, through the nose, or both, and either with or without additional medically suitable compounds. Aerosolized LPEI-NO may also be administered through a medical device such as gas delivery mask or an endotracheal tube used by patients with a tracheostomy. As mentioned above, the greater degree of substitution with $N_2O_2^-$ that is possible with the LPEI-NO of the present invention, compared to previously known branched PEI-NO, allows for delivery

of a therapeutic level of NO to the lungs.

Once administered to a patient, non-fibrous LPEI-NO delivers NO spontaneously under physiological conditions without the need for activation or metabolization. Spontaneous delivery of NO by non-fibrous LPEI-NO also takes place without the creation of free radicals which can injure cells and tissues by oxidation.

It is envisioned that non-fibrous LPEI-NO may be administered to a patient by inhalation for the treatment of pulmonary or other disorders such as, for example, pulmonary hypertension, asthma, and emphysema. In such a case, non-fibrous LPEI-NO is dissolved in a medically appropriate solvent, such as, for example, saline solution, aerosolized by any medically acceptable method, such as, for example, by the use of pressurized gas or a nebulizer or atomizer, and inhaled through the mouth or nose or both, thereby introducing the non-fibrous LPEI-NO into the lungs. Non-fibrous LPEI-NO may also be inhaled through the use of a medical device such as a gas delivery mask or endotracheal tube. While in the lungs, the non-fibrous LPEI-NO releases NO spontaneously, without activation or metabolization, and without the creation of free radical compounds, to treat disorders of the lung such as pulmonary hypertension, asthma, and emphysema. It is well known in the art that NO acts as a vasodilator. When aerosolized non-fibrous LPEI-NO is administered to a patient it may be administered at a rate of about 25 g per adult human patient per 4 hours.

It is also envisioned that non-fibrous LPEI-NO may be administered to a patient topically to treat medical conditions in which an increase blood flow is desired in the area to which the non-fibrous LPEI-NO is applied. It is believed that NO increases localized blood flow in areas to which it is introduced. Non-fibrous LPEI-NO can therefore be used to deliver NO to tissues in which an increased blood flow rate would be desirable. Disorders that may be treated in this manner include both male sexual dysfunction (also known as erectile dysfunction) and female sexual dysfunction. Non-fibrous LPEI-NO used to treat sexual dysfunction may be applied to the genitalia either alone or as a component of, for example, a gel, a cream, an ointment, a spray, a

lubricant, an implant, a patch, or other medical device. When applied, either as a component of one of these products or without additional carrier components, LPEI-NO spontaneously releases NO without activation or metabolization, and without the creation of free radical compounds. NO released by non-fibrous LPEI-NO causes an increase in blood flow to the genitalia, thereby alleviating the symptoms of male or female sexual dysfunction. It is believed that the greater degree of $N_2O_2^-$ substitution with that is possible with the non-fibrous LPEI-NO of the present invention, compared to previously known branched PEI-NO, allows for delivery of an amount of NO sufficient to alleviate symptoms of sexual dysfunction.

In order to demonstrate practice of the present invention, two samples of LPEI, each having a molecular weight of about 450, were converted to LPEI-NO by exposure to NO in methanol/sodium methoxide for approximately 5 days at room temperature and under approximately 100 psi (7.031×10^4 kg/m^2) of pressure. These two samples of LPEI-NO were tested for the amount of substitution occurring at the secondary amines of the LPEI. The degree of substitution is determined by measuring the absorbance of a solution at 252 nm and comparing the absorbance to the absorbance of fully saturated LPEI-NO. Completely saturated LPEI-NO exhibits an absorbance of 6-8 X 10^3 m^{-1}. One sample had an absorbance at 252 nm of 5.2 X 10^3 m^{-1}. The other sample displayed an absorbance of 7.2 X 10^3 m^{-1} at 252 nm, indicating substantially complete modification of the secondary amines of the LPEI.

LPEI-NO with an absorbance at 252 nm = 5.2 X 10^3 m^{-1} was tested in a pulmonary hypertension model. In this model, newborn, random-bred Yorkshire piglets (age 3-5 days) were placed in either normoxic or normobaric hypoxic chambers for 10-12 days. The normobaric, hypoxic chamber was produced by delivering compressed air and nitrogen gas at ratio of 5:4 to either an isolette (Air Shields) or a large incubator. The FiO_2 of the hypoxic chamber was regulated at 0.11 - 0.12. Carbon dioxide was absorbed with soda lime and maintained at 3-5 torr. The temperature of the chamber was controlled at 38°C. The chamber was opened 3-4 times per day to clean the chamber and weigh the animals. The animals were fed *ad libitum* with an artificial sow

milk feeding device attached to the chamber.

On the day of surgery, the animals were weighed and general anesthesia induced using ketamine HCl, Rompin, and atropine. The trachea was intubated and general anesthesia maintained during the surgical preparation using isoflurane, 70% N_2O and 30% O_2. Increased anesthetic medications were provided as needed to optimize pain control. Mechanical ventilatory support was provided using a Servo 900 ventilator (Siemens, Inc.) with a tidal volume of 15 cc/kg and a PEEP of 4 cm H_2O. Minute ventilation and FiO_2 were adjusted to maintain an arterial pCO_2 of 40 torr and pO_2 of 100 torr. Intravenous *d*-tubocurarine was administered intermittently to maintain muscle relaxation. A core temperature of 39.0°C was maintained with a heating pad. Femoral venous and arterial lines were placed by cutdown. Mean arterial pressure was determined using a calibrated transducer attached to the femoral arterial line. During the period of surgical preparation, D5 Ringer's Lactate solution was infused intravenously at 20 cc/kg/hour. During recovery, D5 Ringer's Lactate solution was infused intravenously at 5 cc/kg/hr. A median sternotomy was performed and catheters placed in the main pulmonary artery, left atrium, and right atrium, and secured with purse string sutures. An ultrasonic flow probe (A10, Transonics, Inc. Ithaca, NY) was placed around the pulmonary artery and connected to a small animal flowmeter (T206; Transonics, Inc.).

After a 30 minute period of stabilization, a baseline period followed in which measurements of gas exchange and hemodynamic variables were obtained. Arterial and mixed venous blood gas results were recorded. Peak and end-expiratory ventilatory pressures, and tidal volume measurements were recorded. Oxygenation, intrapulmonary shunt, and lung compliance were calculated using stand formulas. Hemodynamic variables including mean arterial, left atrial, right atrial, and mean pulmonary arterial pressures, along with cardiac output were continuously recorded. Systemic and pulmonary vascular resistance indices were calculated utilizing standard formulas.

Piglets were then randomly assigned under double-blind conditions to

treatment with inhaled nitric oxide (at 20 ppm) or to inhalation by aerosol of non-fibrous LPEI-NO (25 mg or 200 mg). Non-fibrous LPEI-NO was dissolved in saline and used immediately. The percent change from baseline pulmonary vascular resistance, systemic vascular resistance, cardiac output, and PaO_2/FiO_2 ratio was compared over time. After 2 hours, animals were euthanized with an overdose of pentobarbital (50 mg/kg IV push). Changes in the pulmonary vascular resistance (PVRI) and systemic vascular resistance (SVRI) for piglets treated with NO gas or LPEI-NO are summarized in Figure 1.

As shown in Figure 1, non-fibrous LPEI-NO, at a dose of 25 mg or 200 mg per aerosol, produced a significant reduction in pulmonary vascular resistance (PVRI-PEI/NO (25 mg) and PVRI-PEI/NO (200 mg), respectively). This alteration was sustained for the duration of the study (2 hours). LPEI-NO did not substantially alter systemic vascular resistance (SVRI-PEI/NO), indicating that the drug exerts a selective vasodilatory effect on the pulmonary vascular bed. As expected, inhaled NO gas produced a selective vasodilation of the pulmonary vasculature as shown by the reduction in the pulmonary vascular resistance (PVRI-20 ppm NO gas) without a significant alteration of the systemic vascular resistance (SVRI-20 ppm NO gas). The effect of non-fibrous LPEI-NO on pulmonary vascular resistance and was comparable to inhaled NO.

It should be evident that the present invention is highly effective in treating disorders by delivering NO to the patient. It should also be evident that the present invention is highly effective in treating lung disorders by delivering NO to the lungs. It should likewise be evident that the present invention is highly effective in selectively lowering the pulmonary blood pressure of a patient without altering the systemic blood pressure of the patient.

Based upon the foregoing disclosure, it should now be apparent that the use of non-fibrous LPEI-NO will carry out the objects set forth hereinabove. It is, therefore, to be understood that any variations evident fall within the scope of the claimed invention and thus, the selection of specific component elements can be determined without departing from the spirit of the invention herein disclosed and described.

CLAIMS

What is claimed is:

1. A method for the treatment of a patient having, or susceptible to having, symptoms of a disorder or dysfunction comprising:
   administering a non-fibrous linear poly(ethylenimine) diazeniumdiolate to the patient wherein said linear poly(ethylenimine) diazeniumdiolate is capable of releasing a therapeutically effective amount of nitric oxide sufficient to alleviate the symptoms of the disorder or dysfunction.

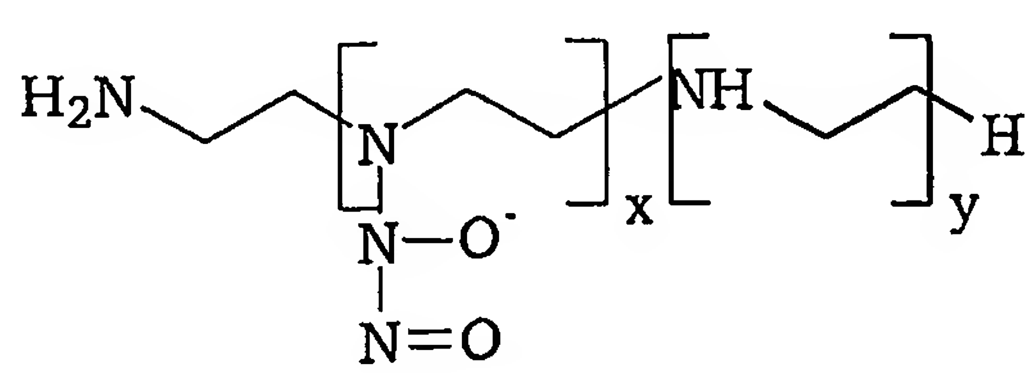
2. The method of claim 1, wherein the linear poly(ethylenimine) component of the non-fibrous poly(ethylenimine) diazeniumdiolate has a number average molecular weight of between about 25,000 and about 200,000.

3. The method of claim 1, wherein the linear poly(ethylenimine) component of the non-fibrous poly(ethylenimine) diazeniumdiolate has a number average molecular weight of less than about 25,000.

4. The method of claim 3, wherein the linear poly(ethylenimine) component of the non-fibrous poly(ethylenimine) diazeniumdiolate has a number average molecular weight of less than about 1,000.

5. The method of claim 1, wherein substantially all of the secondary amine sites of a linear poly(ethylenimine) are substituted with $N_2O_2^-$.

6. The method of claim 1, wherein the linear poly(ethylenimine) diazeniumdiolate is of formula I



(I)

wherein x is the number of $N_2O_2^-$ substituted secondary amines, y is the number of unsubstituted secondary amines, and the ratio of x:y is at least 1.

7. The method of claim 6, wherein the value of x+y is the integer 9 or 10.

8. The method of claim 7, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is in aerosol form.

9. The method of claim 7, wherein the poly(ethylenimine) diazeniumdiolate is administered to a human at a rate of at least 25 g per every four hours.

10. The method of claim 1, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is topically applied to the patient.

11. A method for treating sexual dysfunction comprising the method of claim 10 wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is topically applied to genitalia.

12. The method of claim 11, wherein the sexual dysfunction to be treated is erectile dysfunction.

13. The method of claim 11, wherein the sexual dysfunction to be treated is female sexual dysfunction.

14. A method for delivering nitric oxide to the lungs comprising:

synthesizing a non-fibrous linear poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of $N_2O_2^-$ substituted amines to unsubstituted amines greater than 1.0; and

administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs.

15. The method of claim 14, wherein the step of administering a non-fibrous linear poly(ethylenimine) diazeniumdiolate includes inhaling the linear poly(ethylenimine) diazeniumdiolate into the lungs.

16. A method for treating a human disease selected from the group consisting of pulmonary hypertension, asthma, and emphysema, comprising treating the lungs as set forth in claim 15.

17. The method of claim 14, wherein a majority of the secondary amines of the poly(ethylenimine) are substituted with $N_2O_2^-$.

18. A method for lowering the pulmonary blood pressure of a patient having lungs, without significantly affecting the systemic vascular blood pressure of the patient comprising:

synthesizing a non-fibrous linear poly(ethylenimine) diazeniumdiolate, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate has a ratio of $N_2O_2^-$ substituted amines to unsubstituted amines greater than 1.0; and

administering said non-fibrous linear poly(ethylenimine) diazeniumdiolate to the lungs.

19. The method of claim 18, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is dissolved in saline solution.

20. The method of claim 19, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is aerosolized.

21. The method of claim 20, wherein the non-fibrous linear poly(ethylenimine) diazeniumdiolate is administered to the lungs by inhalation.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/44212

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/785

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 405 919 A (HRABIE JOSEPH A ET AL) 11 April 1995 (1995-04-11) cited in the application column 1, line 9 - line 37 column 9, line 48 -column 10, line 24 claims 1,4,11,22	1-21
X	WO 98 13358 A (BOGDAN CHRISTIAN ;SAAVEDRA JOSEPH E (US); JI XINHUA (US); US HEALT) 2 April 1998 (1998-04-02) claims 1,25,27,79-84	1-21
E	WO 01 92215 A (BONIFANT CHALLICE ;CITRO MICHAEL (US); JI XINHUA (US); BUZARD GREG) 6 December 2001 (2001-12-06) claims 1,2,26,27,55	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 April 2002	15/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stienon, P

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Ir al Application No
PCT/US 01/44212

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 714 511 A (BILLIAR TIMOTHY R ET AL) 3 February 1998 (1998-02-03) cited in the application column 11, line 35 - line 57; claims 1,2	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US 01/44212

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